

FINAL REPORT

Test Facility Study No. 511874

Acute Eye Irritation/Corrosion Study with MLA-3202 in the Rabbit

SPONSOR:

Chempura Corporation
199 Benson Road
MIDDLEBURY, CT 06749
USA

TEST FACILITY:

Charles River Laboratories Den Bosch B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

23 November 2016

Page 1 of 16

TABLE OF CONTENTS

1.	STATEMENT OF GLP COMPLIANCE.....	3
2.	TEST FACILITY QUALITY ASSURANCE STATEMENT	4
3.	SUMMARY	5
4.	INTRODUCTION	6
4.1.	Study Schedule.....	6
4.2.	Purpose.....	6
4.3.	Guidelines	6
4.4.	Retention of Records and Materials.....	6
4.5.	Responsible Personnel	7
4.5.1.	Test Facility	7
4.5.2.	Sponsor Representative.....	7
5.	MATERIALS AND METHODS	8
5.1.	Test Item	8
5.1.1.	Test Item Information	8
5.1.2.	Study Specific Test Item Information.....	8
5.1.3.	Test Item Preparation.....	8
5.2.	Test System.....	8
5.3.	Animal Husbandry	9
5.4.	Weight of Evidence Analysis.....	9
5.5.	Study Design	9
5.6.	Preemptive Pain Management	10
5.7.	Treatment	10
5.8.	Observations	10
5.9.	Interpretation.....	11
5.10.	List of Deviations.....	11
5.10.1.	List of Study Plan Deviations	11
5.10.2.	List of Standard Operating Procedures Deviations.....	11
6.	ELECTRONIC SYSTEMS FOR DATA ACQUISITION.....	11
7.	RESULTS.....	12
7.1.	Irritation	12
7.2.	Corrosion.....	12
7.3.	Coloration / Remnants	12
7.4.	Toxicity / Mortality.....	12
8.	CONCLUSION	12
LIST OF APPENDICES		
APPENDIX 1	TABLES	13
APPENDIX 2	TEST ITEM CERTIFICATE OF ANALYSIS.....	15

1. STATEMENT OF GLP COMPLIANCE

Charles River Den Bosch, 's-Hertogenbosch, The Netherlands

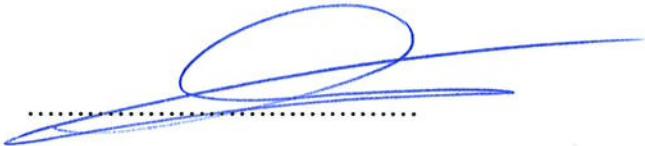
All phases of this study performed by the test facility were conducted in compliance with:

- OECD Principles of Good Laboratory Practice;
- EC Council Directive 2004 (2004/10/EC, February 11, 2004, Official Journal of February 20, 2004).

The data generated and reported are considered to be valid.

Charles River Den Bosch

Signature:



Name: A.H.B.M. van Huygevoort, MSc.

Title: Study Director

Date: 23 November 2016

2. TEST FACILITY QUALITY ASSURANCE STATEMENT

Charles River Den Bosch, 's-Hertogenbosch, The Netherlands.

Study title: Acute eye irritation/corrosion study with MLA-3202 in the rabbit

This report was inspected by the Charles River Den Bosch Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s). The reported method and procedures were found to describe those used and the report reflects the raw data. During the on-site process inspections, procedures applicable to this type of study were inspected. The dates of Quality Assurance inspections are given below.

Project 511874

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date to TFM and SD*
Study	Study Plan Report	08-Aug-2016 03-Nov-2016	08-Aug-2016 03-Nov-2016	08-Aug-2016 03-Nov-2016
Process	Animal Facilities Test Substance Handling Exposure Observations/Measurements Specimen Handling	04-Jul-2016	15-Jul-2016	22-Jul-2016
	Test Substance Receipt Test Substance Handling	22-Aug-2016	02-Sep-2016	09-Sep-2016
	Test Substance Formulation Test Substance Handling	24-Aug-2016	05-Sep-2016	08-Sep-2016

*TFM=Test Facility Management SD = Study Director

The facility inspection program is conducted in accordance with Standard Operating Procedure.

The review of the final report was completed on the date of signing this QA statement.

Charles River Den Bosch

Signature:

Name: **Ulrich Wiets**
Quality Assurance Auditor

Date:

3. SUMMARY

Acute eye irritation/corrosion study with MLA-3202 in the rabbit.

The study was carried out based on the guidelines described in:
OECD No.405 (2012) "Acute Eye Irritation / Corrosion"
EC, No 440/2008, B5: "Acute Toxicity: Eye Irritation/Corrosion"
EPA, OPPTS 870.2400 (1998): "Acute Eye Irritation"
JMAFF Guidelines (2000), including the most recent revisions.

Single samples of 0.1 mL of MLA-3202 were instilled into one eye of each of three rabbits.
Observations were made 1, 24, 48 and 72 hours after instillation.

Instillation of MLA-3202 into one eye of each of three rabbits resulted in irritation of the conjunctivae, which consisted of redness, chemosis and/or discharge. The irritation had completely resolved within 24 hours in two animals and within 48 hours in the other animal.

Based on these results, MLA-3202 does not have to be classified and has no obligatory labelling requirement for eye irritation according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments) and EC criteria for classification and labelling requirements for dangerous items and preparations (Council Directive 67/548/EEC) (including all amendments).

4. INTRODUCTION

4.1. Study Schedule

Experimental starting date : 23 August 2016
Experimental completion date : 08 September 2016

4.2. Purpose

The purpose of this acute eye irritation/corrosion study was to assess the possible irritation or corrosion potential when a single dose of the test item was placed in the conjunctival sac of the rabbit eye.

This study should provide a rational basis for risk assessment in man.

The absence of eye pigmentation in the albino rabbit facilitates the evaluation of induced eye reactions. The ocular route was selected because the test item may accidentally come into contact with the eyes during manufacture, handling and/or use.

4.3. Guidelines

This type of study plan was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee (DEC 14-20) as required by the Dutch Act on Animal Experimentation (February 1997).

The study procedures described in this report were in compliance with the following guidelines:

- Organization for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.405, "Acute Eye Irritation / Corrosion", Paris, 2012.
- Commission Regulation (EC) No 440/2008 Part B: Methods for the Determination of Toxicity and other Health Effects; B5: "Acute Toxicity: Eye Irritation/Corrosion". Official Journal of the European Union No. L142, May 2008, including most recent amendments.
- United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation. Office of Prevention, Pesticides and Toxic Items (7101), EPA 712-C-98-195, August 1998.
- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), 12 Nousan, Notification No 8147, November 2000; including the most recent partial revisions.

4.4. Retention of Records and Materials

Records and material pertaining to the study, which include study plan and amendments, raw data, specimens, except perishable specimens, and the final report will be retained in the archives of the test facility for a minimum of 5 years after the finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. The test facility will retain information concerning decisions made.

A sample of the test item will be retained until expiry date or applicable retest date. After this period the sample(s) will be destroyed.

4.5. Responsible Personnel**4.5.1. Test Facility**

Study Director A.H.B.M. van Huygevoort, MSc.
Coordinating Biotechnician R. Eyndhoven (Charles River Den Bosch)
QA C.J. Mitchell, BSc. (Charles River Den Bosch)
christine.mitchell@crl.com
Test Facility Management H.H. Emmen, MSc. (Charles River Den Bosch)
Representative harry.emmen@crl.com

4.5.2. Sponsor Representative

Study Monitor Audrey Batoon, Ph.D.

5. MATERIALS AND METHODS

5.1. Test Item

5.1.1. Test Item Information

Test item	207258/A
Identification	MLA-3202
Appearance	Clear amber-red liquid
Batch	RC-1045
Purity/Composition	UVCB
Test item storage	At room temperature
Stable under storage conditions until	17 February 2019 (expiry date)

5.1.2. Study Specific Test Item Information

Quality system of the CoA	GLP
Purity/composition correction factor	No correction factor required
Test item handling	No specific handling conditions required
Stability at higher temperatures	Stable
Chemical name (IUPAC), synonym or trade name	Amides, tallow, N,N-bis(2-hydroxypropyl)
CAS Number	1454803-04-3
pH	6-7
Specific gravity/density	0.9394

5.1.3. Test Item Preparation

The test item was instilled undiluted as delivered by the Sponsor. No correction was made for the purity/composition of the test item, since the guidelines require a fixed amount to be instilled.

5.2. Test System

Species	Albino rabbit, New Zealand White, (SPF-Quality). Recognized by international guidelines as the recommended test system (e.g. EC, OECD) Source: Charles River France, L'Arbresle, France
Number of animals	3 Males
Age and body weight	At start of dosing, the animals were between 12 and 24 weeks old and body weights were at least 1.5 kg.
Identification	Earmark.
Health inspection	At least prior to dosing. It was ensured that the animals were healthy and that the eyes were free from any abnormality.

5.4. Animal Husbandry

Conditions

Environmental controls for the animal room were set to maintain 18 to 24°C, a relative humidity of 40 to 70%, at least 10 air changes/hour, and a 12-hour light/12-hour dark cycle. Any variations to these conditions were maintained in the raw data and had no effect on the outcome of the study.

Accommodation

Animals were individually housed in labeled cages with perforated floors (Eboco, Germany, dimensions 67 x 62 x 55 cm) and shelters (Eboco, Germany, dimensions 40 x 32 x 23 cm). Acclimatization period was at least 5 days before start of treatment under laboratory conditions.

Diet

Pelleted diet for rabbits (Global Diet 2030 from Harlan Teklad®, Mucedola, Milanese, Italy) approximately 100 grams per day. Hay (TecniLab-BMI BV, Someren, The Netherlands) and wooden sticks (Swedish aspen wood, Bioservices, Uden, The Netherlands) were available during the study period.

Water

Free access to tap water.

Diet, water, bedding and cage enrichment evaluation for contaminants and/or nutrients was performed according to facility standard procedures. There were no findings that could interfere with the study.

5.5. Weight of Evidence Analysis

In the interest of animal welfare and to minimize any testing likely to produce severe responses in animals, a weight of evidence analysis was performed, prior to the start of this *in vivo* eye irritation study in the rabbit. As recommended in the test guidelines, all available information was evaluated (e.g. existing human and animal data, literature, item data supplied by the Sponsor, analysis of structure activity relationships (SAR), physicochemical properties and reactivity (pH, buffering capacity) and *in vitro*, *ex vivo* and *in vivo* tests) to determine the need for *in vivo* eye testing.

The *in vitro* Bovine Corneal Opacity and Permeability (BCOP) study (project 514868) showed that the test item was non-irritating. This negative BCOP result should be confirmed in an *in vivo* eye irritation study. Based on the available information, it was concluded that there was the need to perform this *in vivo* eye irritation study in rabbit in order to establish the possible eye irritating properties of the test item.

5.6. Study Design

The study was performed in a stepwise manner and was started by treatment of a single rabbit (sentinel). The two other animals were treated in a similar manner 1 week later, after considering the degree of eye irritation observed in the first animal.

5.8. Preemptive Pain Management

One hour prior to instillation of the test item, buprenorphine (Buprenodale®, Dechra Ltd., Stoke-on-Trent, United Kingdom) 0.01 mg/kg was administered by subcutaneous injection in order to provide a therapeutic level of systemic analgesia.

Five minutes prior to instillation of the test item, two drops of the topical anesthetic alcaine 0.5% (SA Alcon-Couvreur NV, Puurs, Belgium) were applied to both eyes.

5.9. Treatment

Each animal was treated by instillation of 0.1 mL of the test item, in the conjunctival sac of one of the eyes after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test item. The other eye remained untreated and served as the reference control.

Immediately after the 24-hour observation, a solution of 2% fluorescein (Merck, Darmstadt, Germany) in water (adjusted to pH 7.0) was instilled into both eyes of each animal to quantitatively determine corneal epithelial damage. Any bright green stained area, indicating epithelial damage, was estimated as a percentage of the total corneal area.

After the final observation, the animals were sacrificed by intra-venous injection of Euthasol® 20% (AST Farma BV, Oudewater, The Netherlands).

5.10. Observations

Mortality/Viability	Twice daily.
Toxicity	At least once daily.
Body Weight	Day of treatment (prior to instillation) and after the final observation.
Irritation	The eyes of each animal were examined approximately 1, 24, 48 and 72 hours after instillation of the test item. The irritation scores and a description of all other (local) effects were recorded.

The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given were recorded:

CORNEAL IRRITATION

Opacity: degree of density (area most dense taken for reading)

No ulceration or opacity (may include slight dulling of normal luster).....	.0
Scattered or diffuse areas of opacity, details of iris clearly visible.....	.1
Easily discernible translucent area, details of iris slightly obscured.....	.2
Nacreous area, no details of iris visible, size of pupil barely discernible3
Opaque cornea, iris not discernible through the opacity4

Area of cornea involved:

No ulceration or opacity0
One quarter or less but not zero1
Greater than one quarter, but less than half2
Greater than half, but less than three quarters.....	.3
Greater than three quarters, up to whole area4

IRIS

Normal0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive)1
No reaction to light, hemorrhage, gross destruction (any or all of these)2

CONJUNCTIVAL IRRITATION

Redness (refers to palpebrae and sclera, excluding cornea and iris):

Blood vessels normal0
Some blood vessels definitely hyperaemic (injected).....	.1
Diffuse, crimson color, individual vessels not easily discernible2
Diffuse beefy red3

Chemosis (refers to lids and/or nictitating membranes):

No swelling0
Any swelling above normal (includes nictitating membranes)1
Obvious swelling with partial eversion of lids2
Swelling with lids about half closed3
Swelling with lids more than half closed.....	.4

Discharge:

No discharge (may include small amounts observed in inner canthus of normal animals) 0
 Any amount different from normal and/or lacrimation 1
 Discharge with moistening of the lids and hairs just adjacent to lids 2
 Discharge with moistening of the lids and hairs (considerable area around the eye) 3

Where standard lighting was considered inadequate for observing minor effects, eye examinations were performed using an ophthalmic examination lamp.

Necropsy No necropsy was performed according to study plan.

5.11. Interpretation

The results were evaluated according to:

- Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments).
 - Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of items and mixtures (including all amendments).

5.12. List of Deviations

5.12.1. List of Study Plan Deviations

1. Deviations from the maximum level of daily mean relative humidity occurred.
Evaluation: Laboratory historical data do not indicate an effect of the deviations.

The study integrity was not adversely affected by the deviation.

5.12.2. List of Standard Operating Procedures Deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

6. ELECTRONIC SYSTEMS FOR DATA ACQUISITION

The following electronic system was used for data acquisition:

- REES Centron Environmental Monitoring system version SQL 2.0 (REES scientific, Trenton, NJ, USA).

7. RESULTS

For detailed results see [APPENDIX 1](#).

7.1. Irritation

Instillation of approximately 0.1 mL of MLA-3202 into one eye of each of three rabbits resulted in irritation of the conjunctivae, which consisted of redness, chemosis and/or discharge. The irritation had completely resolved within 24 hours in two animals and within 48 hours in the other animal.

No iridial irritation or corneal opacity were observed, and treatment of the eyes with 2% fluorescein 24 hours after test item instillation revealed no corneal epithelial damage.

7.2. Corrosion

There was no evidence of ocular corrosion.

7.3. Coloration / Remnants

No staining of (peri) ocular tissues by the test item was observed and no test item remnants were seen.

7.4. Toxicity / Mortality

No signs of systemic toxicity were observed in the animals during the test period and no mortality occurred.

8. CONCLUSION

Based on these results, MLA-3202 does not have to be classified and has no obligatory labelling requirement for eye irritation according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments) and EC criteria for classification and labelling requirements for dangerous items and preparations (Council Directive 67/548/EEC) (including all amendments).

APPENDIX 1
TABLES

Table 1: Individual Eye Irritation Scores

Animal	Time after dosing	Cornea			Iris	Conjunctivae			Comments
		Opacity (0-4)	Area (0-4)	Fluor area (%) ²	(0-2)	Redness (0-3)	Chemosis (0-4)	Discharge (0-3)	
164¹	1 hour	0	0		0	1	0	0	-
	24 hours	0	0	0	0	0	0	0	-
	48 hours	0	0		0	0	0	0	-
	72 hours	0	0		0	0	0	0	-
175	1 hour	0	0		0	1	0	1	-
	24 hours	0	0	0	0	0	0	0	-
	48 hours	0	0		0	0	0	0	-
	72 hours	0	0		0	0	0	0	-
178	1 hour	0	0		0	1	1	1	-
	24 hours	0	0	0	0	1	0	0	-
	48 hours	0	0		0	0	0	0	-
	72 hours	0	0		0	0	0	0	-

¹ Sentinel, ² Green staining after fluorescein treatment (percentage of total corneal area)

Table 2: Mean Value Eye Irritation Scores

Animal	Mean 24, 48 and 72 hours			
	Corneal opacity	Iris	Conjunctivae	
			Redness	Chemosis
164	0.0	0.0	0.0	0.0
175	0.0	0.0	0.0	0.0
178	0.0	0.0	0.3	0.0

Table 3: Animal Specifications

Animal	Sex	Age at start (weeks)	Body weights (grams)	
			prior to application	at termination
164	♂	13	3052	3088
175	♂	13	2421	2670
178	♂	13	2671	2769

APPENDIX 2
TEST ITEM CERTIFICATE OF ANALYSIS



Chemtura Corporation
12 Spencer St
Naugatuck, CT 06770

Analytical Services
www.chemtura.com

Certificate of Purity

Customer: Support for Toxicology Studies

Test Substance Name: MLA3202; Amides, tallow, N,N-bis(2-hydroxypropyl)

Physical Appearance: Liquid

CAS No.: 1454803-04-3

Ref. or Lot Number: RC-1045

Date of Analysis: revised March 18, 2016 (original issue March 7, 2016)

Percent Composition	Monoisotopic Mass (daltons)	Formula	Structure/ Identity
33.1	397.4	C ₂₄ H ₄₇ NO ₃	C18:1 (oleic) tallow amides, N,N-bis(2-hydroxypropyl)
22.9	371.3	C ₂₂ H ₄₅ NO ₃	C16:0 (palmitic) tallow amides, N,N-bis(2-hydroxypropyl)
13.6	395.4	C ₂₄ H ₄₅ NO ₃	C18:2 (linoleic) tallow amides, N,N-bis(2-hydroxypropyl)
11.0	399.4	C ₂₄ H ₄₅ NO ₃	C18:0 (stearic) tallow amides, N,N-bis(2-hydroxypropyl)
6.0	369.3	C ₂₂ H ₄₃ NO ₃	C16:1 (palmitoleic) tallow amides, N,N-bis(2-hydroxypropyl)
3.2	419.3	C ₂₆ H ₄₅ NO ₃	C20:4 (eicosatetraenoic) tallow amides, N,N-bis (2-hydroxypropyl)
2.0	393.3	C ₂₄ H ₄₃ NO ₃	C18:3 (linolenic) tallow amides, N,N-bis(2-hydroxypropyl)
1.5	282.3	C ₁₈ H ₃₄ O ₂	C18:1 (oleic) acid
1.1	421.4	C ₂₆ H ₄₇ NO ₃	C20:3 (eicosatrienoic) tallow amides, N,N-bis (2-hydroxypropyl)
5.6			Sum of residual components (< 1% each)
100.0			Total

Blake Lewis 3/7/16
 Blake Lewis Date
 Analytical REACh Scientist, Analytical Services

Colin Moore 3/7/16
for AJN Date
 Albert J. Nitowski
 Sr. Technology Manager
 Analytical and Lab Support Services